Quick Scoping Review (QSR): What methods are currently available for the quantitative detection of infectious human viruses in bathing waters?

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Executive summary

Background

The EU Commission aims to review the existing Bathing Waters Directive (2006/7/EC) by 2020. The inclusion of monitoring for human enteric viruses in the Directive might be considered at that time. For this reason, there is a desire to know what methods are currently available for the quantification of infectious human viruses in bathing waters. Since not all viruses detected in water bodies will be capable of infection, any such future guidelines may need to be able to distinguish between detection and infectivity to avoid over-estimation of the risks to human health. Moreover, accurate determination of risk will ensure that mitigating measures to lower the risk are proportionate and cost effective.

Quick Scoping Review (QSR) process

The objective of this QSR was to review and summarize the methods currently available for the quantitative detection of infectious viruses that would be suitable for use in the analysis of bathing waters. We started with a meeting between Defra, the reviewers and a number of stakeholders, when the overall approach was agreed. This was followed by production of a QSR methodology document, outlining the sources of information to be reviewed and the approach for identifying the relevant information within these sources. It was agreed the QSR would include keyword 'searches' in: peer-review literature, UK research grant proposals, international patents and International Organisation for Standardization (ISO) documents. It was also agreed that assessment of methods for the concentration of viruses in water samples (prior to any detection or quantification) would remain outside the scope of this QSR.

Key findings

The QSR has revealed that current scientific literature on the recovery of infectious human viruses in water samples focused on a relatively small number of viruses, limited largely to: human adenovirus, norovirus, enterovirus, poliovirus and influenza A virus. The most common water sources from which viruses were recovered and studied were: wastewater (effluent), surface water (rivers, lakes), and sea water.

Our review of existing methods for the detection and quantification of infectious human enteric viruses in water indicated that techniques fall within two main categories:

- Cell culture-based methods, which measure infectious virus through the direct infection of a limited range of human or mammalian host cells. Several novel methods have been developed to determine whether a virus has indeed infected a host cell, many now relying on one or more molecular-based techniques.

And,

- Structural integrity methods, which are based on the assumption that a virus is infectious if it has a structurally intact capsid, antigen (i.e., attachment site on viral capsid used for binding to the host cell) and/or genome (i.e., DNA or RNA). Conclusions from studies using structural integrity techniques are substantially strengthened when more than one viral structure was assayed, most commonly the capsid or antigen and genome integrity.

Implications for policy and for further research

The use of structural integrity methods for the detection and quantification of infectious human enteric viruses compares favourably to cell culture-based methods on: cost, labour, equipment, safety, breadth (i.e., suitable for any virus) and scalability (i.e., easily adaptable to varying study size). However, structural integrity techniques only allow inference of infectivity—they do not demonstrate the actual infection of a human or mammalian cell. Furthermore, most existing studies employing structural integrity techniques have not validated the techniques against cell culture. Cell culture-based techniques are the only available methods that can be relied upon as a direct and validated measure of infectivity, which is an important criterion when considering public health. No such validation or statistical measure of protection can be provided using structural integrity methods at present.

There is a clear need for further research into the question of viral infectivity, with a focus on standardising and streamlining methods to facilitate comparisons between different water sources, over time, space and between research groups. Moreover, nearly all studies reviewed differ in the sample concentration steps used prior to the measure of infectivity. The conclusions from virus infectivity studies or monitoring campaigns will continue to be difficult to interpret across time and space without any standard methods (e.g., ISO) for concentrating different viruses from varying waters sources.

Finally, new methods need to be developed that aim to maximise the scalability of determining viral infectivity (i.e., low cost, high throughput), while also providing conclusions that are relevant to public health. The application of novel molecular tools, synthetic biology and ‘lab-on-a-chip’ approaches might be among the more promising sources of innovation looking forward. In the short term, however, structural integrity assays might prove valuable as a quick screening tool to identify polluted waters, which can be followed up with cell-culture methods for determining quantitative measures of infectious virus. Concurrently, efforts should be made to validate structural integrity assays against an appropriate cell culture-based method to strengthen the evidence base for their use in the assessment and protection of public health in bathing waters.

Background

The EU Commission will be reviewing the Bathing Waters Directive (2006/7/EC) by 2020 and may consider inclusion of monitoring for human enteric viruses in future updates to the Directive. For this reason, there is a desire to know what methods are currently available for the detection and quantification of infectious human viruses in bathing waters. Since not all viruses detected in water bodies will be capable of infection, any such future guidelines may need to be able to distinguish between detection and infectivity to avoid over-estimation of the risks to human health. Moreover, accurate determination of risk will ensure that mitigating measures to lower the risk are proportionate and cost-effective.

Objective of the Quick Scoping Review (QSR)

The objective of this quick scoping review was to collate and summarise the scale and scope of research evidence available in response to the primary question. This summary of research and knowledge can be used to start to answer the questions posed and to inform requirements for future evidence synthesis and primary research.

Primary Question

This study aimed to find and collate research that addressed the following question: What methods are currently available for the quantification of infectious human viruses in bathing waters? By 'currently available methods' we are referring to the peer-reviewed literature, International Organisation for Standardization (ISO) standards and patents.

PICO elements

In order to better understand the question, it was broken down into constituent parts. This QSR was guided by the PICO approach (population, intervention, comparator and outcome). The elements relating to this QSR are show in Table 1.

Table 1. Population, intervention, comparator and outcome (PICO) elements relating to the question 'What methods are currently available for the quantitative detection of infectious human viruses in bathing waters?'

PICO element	PICO element within this QSR
Population	Infectious human viruses in inland and coastal waters
Impact	Method selection
Control	Cell lines that are susceptible to infection by the target virus
Outcome	Detection or determination of infectivity

Methods

The method used in the development of this QSR was based on the four stages of QSR described by draft Defra guidance for the production of QSRs and rapid evidence assessments (Collins *et al.*, 2014). The four stages are: searching for evidence (develop and test a search strategy, identify keywords and points of reference, identify relevant information sources, apply research strategy); refining and storing evidence; knowledge mapping (develop a conceptual framework to include policy drivers and primary question); and communicate findings.

Search strategy

An initial scoping search was performed to validate the search keywords and methodology. Search terms were formulated by the review team in collaboration with the Steering Group (Table 2). These terms were tested for specificity and sensitivity using a structured search within the online databases: Web on Knowledge and Pubmed. The volume of relevant literature was used to gauge the effectiveness of each of the search terms and their syntax in the search string. Selected key papers were used as a guide to further assess the efficacy of different search strategies.

Keywords

All the keywords found in Table 2 were explored in varying combinations as part of the search strategy. The final keywords and syntax used for the Pubmed and Web of Science (WoS) search was:

<line 1> (detection or detect or monitor or monitoring or extract or extraction) AND

<line 2> (infectious or infective or infectivity or viable or inactiv* or intact or integrity) AND

<line 3> (virus or viral or phage) AND

<line 4> water

Note: **<line x>** is for illustrative purposes, not part of the search expression.

The search algorithm above is exactly as used for searching in Pubmed. All keywords denoted in brackets for the Pubmed Search, were prefaced using 'TS=', which is the required WoS database term 'Topic Search', i.e., TS=(keywords) AND TS=(keywords), thereby providing an identical search algorithm across both databases.

The first line of the search expression refers to the *recovery of the virus*, with the second line referring to the *nature of the virus*, the third line referring to the *virus itself* and the last line referring to the water source from which it was recovered. Notably, phage was included in the third line not because phage are among our inclusion criteria, but because many papers that discuss 'phage' also discuss relevant viruses to this QSR. Where the paper only discusses phage and not a human infectious virus, the paper was eventually excluded. A wildcard (*) was required for the term 'inactiv*' so as to capture multiple word endings, e.g., inactive, inactivity, inactivated. Other words included as our search terms were also examined using the wildcard, but they did not improve the overall search efficacy beyond what could be achieved by utilising two variants of the same root, i.e., (detect and detection) and (monitor and monitoring).

The European Patents Office database (containing worldwide patents) http://worldwide.espacenet.com/advancedSearch?locale=en_EP was searched to reveal patent submissions and the ISO catalogue relating to the microbiology of waters was reviewed (http://www.iso.org/iso/iso_catalogue/catalogue_ics/catalogue_ics_browse.htm?ICS1=07&ICS2=100&ICS3=20) to identify relevant ISO documents. Grants on the Web (http://gow.epsrc.ac.uk/) and (http://gotw.nerc.ac.uk/) was searched to examine whether there were any relevant studies in progress on this topic in the UK. No further grey literature was surveyed.

Table 2. Keywords and qualifiers to be used in the literature search

Virus keywords	Virus-specific alternatives (to be combined with virus)	Water keywords (or words to be used in combination with water)	Methodology-associated keywords	Other
Virus	Human	Water	Detection	Risk
Viral	Enteric	Bathing	Recovery	Directive
Infectious* virus	Gastrointestinal*	Recreational	Quantification	Surveillance
Infective* virus	Intestinal	Swimming	Assessment	Monitoring
Active virus	Gut	Fresh	Isolation	
Inactive virus	Respiratory	Marine	Extraction	
Intact virus		Estuary		
		Lake		
		Inland		
		Coastal		
		Surface water		
		Saltwater		
		Groundwater		
		Springs		
		Sewage effluent		
		Wastewater		

Search sources

As noted above, peer-reviewed literature was recovered using the WoS and PubMed databases.

Study inclusion criteria

All retrieved studies were assessed for relevance using the following inclusion criteria:

Relevant subjects: Studies that investigate the recovery of human infectious viruses from all water sources and also employ methods for the quantification or estimation of infectious human virus.
Geographical Reference: No geographical limitations.
Climatic conditions: All
Language: Studies published in English.
Date: Publication date between 1 January 2010 and 1 January 2015. A start date of 2010 was chosen as it was intended to focus the QSR on the most recent research developments. It was assumed that any efficacious method published prior to 2010 would have been replicated in the literature within this QSR's five-year timeframe.
Population: All infectious human viruses.
Impact: Method selection.
Outcome: Detection of infectivity or measures of structural integrity.

Peer-Reviewed article screening

The inclusion criteria were applied to all articles at the title and abstract level (Figure 1). Studies that passed the inclusion criteria were imported into a unique EndNote X7™ library. Duplicates were removed using the automatic function in the EndNote X7™ software. A record of each search was made to enable a re-run of the search if needed. The following data were recorded: date the search was conducted; database name; search term; number of hits; notes. The final EndNote X7™ outputs were extracted in Excel, so that they are accessible to a wider audience.

Studies passing the initial inclusion criteria, but failing upon subsequent reading of the abstract and paper were recorded and stored in a unique EndNote X7™ library subfolder. The inclusion criteria were applied by one reviewer to all articles. *Review Articles* were excluded, but were used to verify that the search terms captured all of the relevant articles within the date restricted period. Theoretical and conceptual studies were included in this review if they presented feasible 'ways forward' addressing our primary question.

Patent, ISO and Grants screening

A search/review of the:

- European Patents Office;

- ‘microbiology of waters’ ISO list
- Engineering and Physical Sciences Research Council (EPSRC) and
- Natural Environment Research Council (NERC) proposals database

was additionally undertaken. The methodology and results for these searches are provided in Appendix B. The above sources of information did not provide any results of relevance to this study or any results which were not already covered in the literature reviewed.

Results

The total search hits and subsequent relevant results from the review of peer-review literature are shown in Figure 1. The combined WoS and Pubmed results amounted to 816 articles that were screened at the ‘Title’ level. Following the ‘Title’ search, 120 articles were passed on to the “Abstract” screening stage. All remaining articles after Abstract screening (59) were subsequently read and assessed for inclusion/exclusion. The references within each of the papers resulting from the ‘Article’ screen (49) were assessed for any missing and relevant articles. One additional reference was added to the final list of included articles, as reflected in Figure 1. A total of 50 articles were used to populate the extraction tables, summarised in Figures 2, 3, and 4.

The review of patents, ISO documents, EPSRC and NERC grant proposals (Appendix B) did not provide any results of relevance to this study. The exception to this is a large study, with multiple partners, titled “New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum”; this study will commence in April 2015 (running until 2018), hence its outputs are not available yet and cannot be included in this review. After contacting the investigators of this project, it was made clear that there was to be no measure of infective virus in this study.

A total of 31 different viruses (including the different strains of virus) were examined within the 50 research articles used for this review (Appendix A: Table A1), with the majority of studies investigating one or more of the following viruses: human adenovirus (n=25), poliovirus (n=11), enterovirus (n=9), norovirus (n=8) and avian influenza virus (n=7; see Figure 2 and Appendix A, Tables A1, A4). In accordance with our methodology, only strains of virus that infect humans were included in this review, with two exceptions. Avian influenza was included as there is the potential for human infection, and the simian rotavirus

SA11 (group A, simian type G3) was also included only because the authors of the paper utilised a unique methodology not covered by other papers—a methodology applicable to all relevant viruses (Yang *et al.*, 2011). All other non-human infectious viruses were excluded. A method has recently been developed for the culture of human norovirus (Jones *et al.*, 2014), which, if repeatable, might prove applicable to the quantitative detection of infectious norovirus in bathing waters, but was too recent of a development to impact this review.

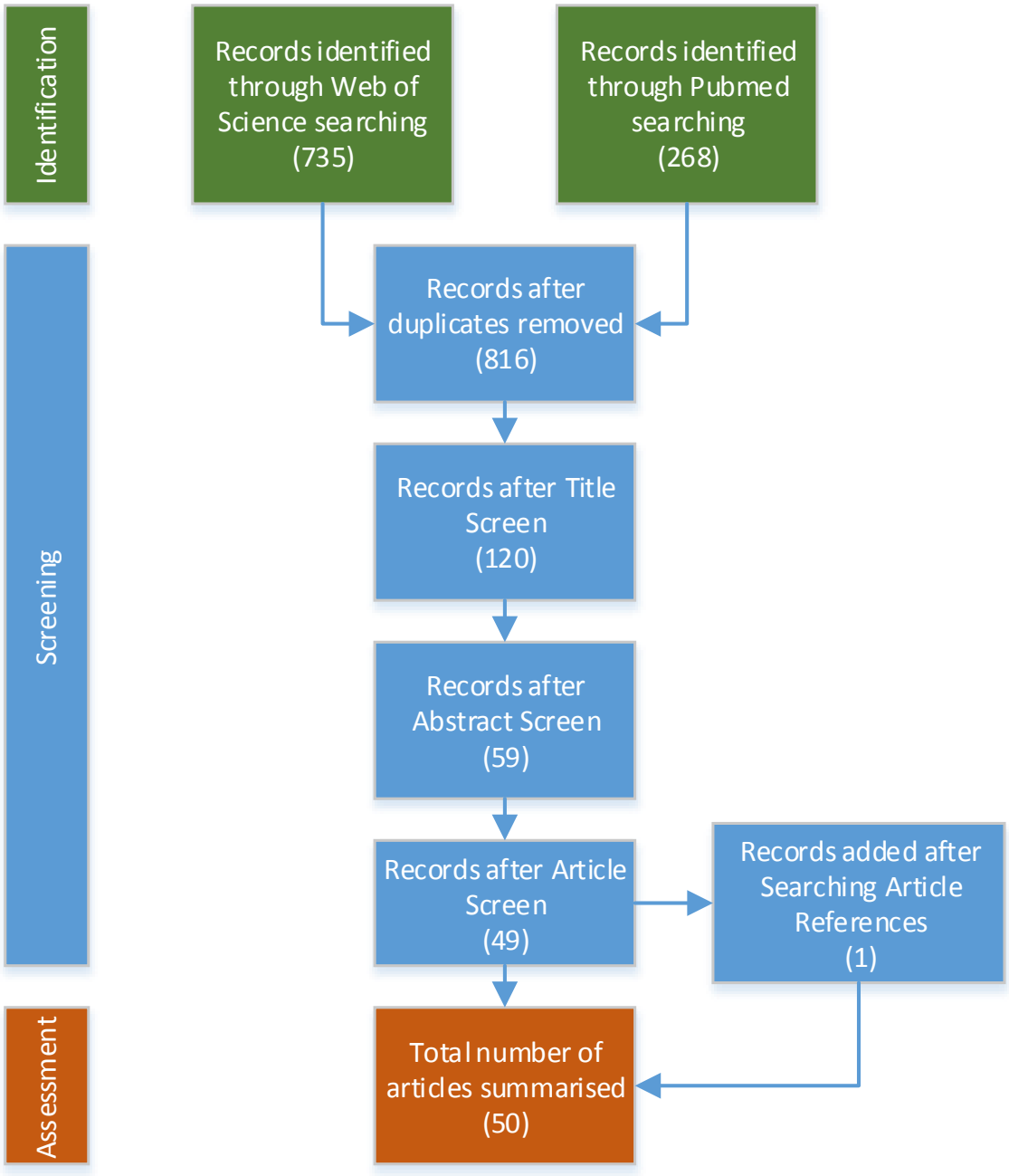

Figure 1. Map of the research collation and screening process

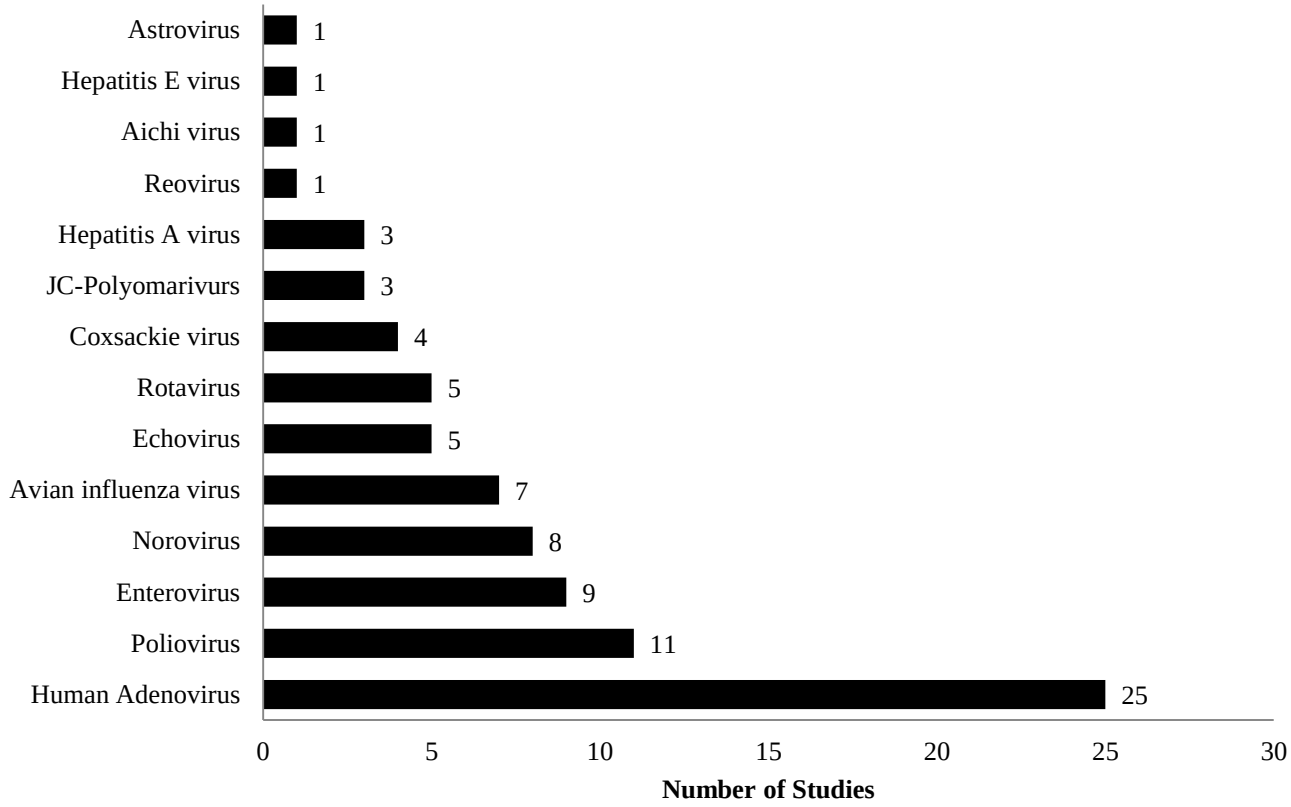

Figure 2. Human infectious viruses in the literature reviewed, including studies from both cell culture-based methods and structural integrity assays. Multiple viruses per research article were common.

The majority of studies reviewed employed a cell culture-based approach (Figure 3; Appendix A, Table A2), which is comprised of three main methods:

1) standard plaque assay (n=10, i.e., number of studies employing this method);
2) cell culture with cytopathic effects as an end-point i.e., the determination of virus infection from visual changes in the host cell (n=13); and
3) integrated cell culture followed by a polymerase chain reaction (ICC-PCR) step, i.e., the determination of virus infection using molecular methods (n=20).

Nine additional studies using cell culture-based methods employed antibodies, dyes or fluorescent reporters to interact with novel structures within the virus-infected cell. These nine studies were categorised as 'non-PCR infection reporters' (see Appendix A: Table A2). An additional study used human volunteers to determine the length of time norovirus can remain in water and maintain its infectivity (Seitz *et al.*, 2011).

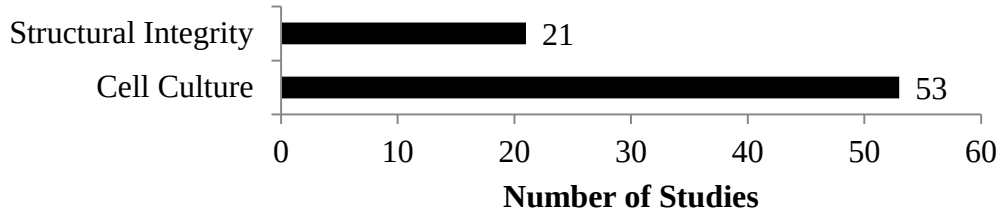

Figure 3. Number of studies that determine viral infectivity by one of two broad methods. Multiple methods per research article were common.

Interrogating the structural integrity of the viral capsid, antigens and/or genome comprises a second strategy for assessing viral infectivity. These methods are infrequently used in conjunction with cell culture-based methods, as they are typically employed as an inexpensive, rapid, high throughput method to circumvent the need for cell culture. Such methods offer a measure of virus within a sample with the potential to infect humans. Viral capsid integrity assays (n=20) are the most commonly employed structural integrity assays. The majority of these assays challenge the virus with degradative enzymes, such as RNAse or DNAse, the effects of which would remove all exposed viral genomes, i.e., not fully protected by an intact viral capsid. Immunofluorescent markers, typically linked to an antigen of a component of the capsid, offer evidence of virus capsid and/or receptor integrity. Viral genome integrity assays have been achieved primarily through the use of PCR, where the varying success of each of the primers used would lend insight into the integrity of the genome. The majority of studies reviewed in this QSR that employed a structural integrity-based method of infectivity used an initial enzyme treatment to degrade the unprotected viral genome followed by the use of immunofluroscent antibodies to verify the integrity of the viral coat antigens. Cell-binding reverse transcriptase (RT)-PCR is a novel method that utilises the binding of a virus to a host cell to confirm structural integrity, followed by PCR to confirm genome integrity (Tian *et al.*, 2012; Wang *et al.*, 2014). This method is the basis of a new patent application in the United States for the detection of infectious norovirus (http://www.freepatentsonline.com/8993229.pdf), but as the online publication date was out of scope for this QSR and it was not submitted to the European Patents Office, it was not formally included in this QSR.

A range of human and mammalian cell lines (n=15) were used for the cell culture method (see Figure 4; two chicken-based cell lines were used for influenza virus culture, i.e., CRBC

and embyronated egg). The predominant use of A549 and BGM cell lines reflects their suitability for culturing adenovirus, enterovirus and poliovirus - the most commonly investigated viruses. A range of additional cell lines were employed as noted in Figure 4 and Appendix A: Table A4, the most frequent of these being: 293, RD, MA104 and Caco-2.

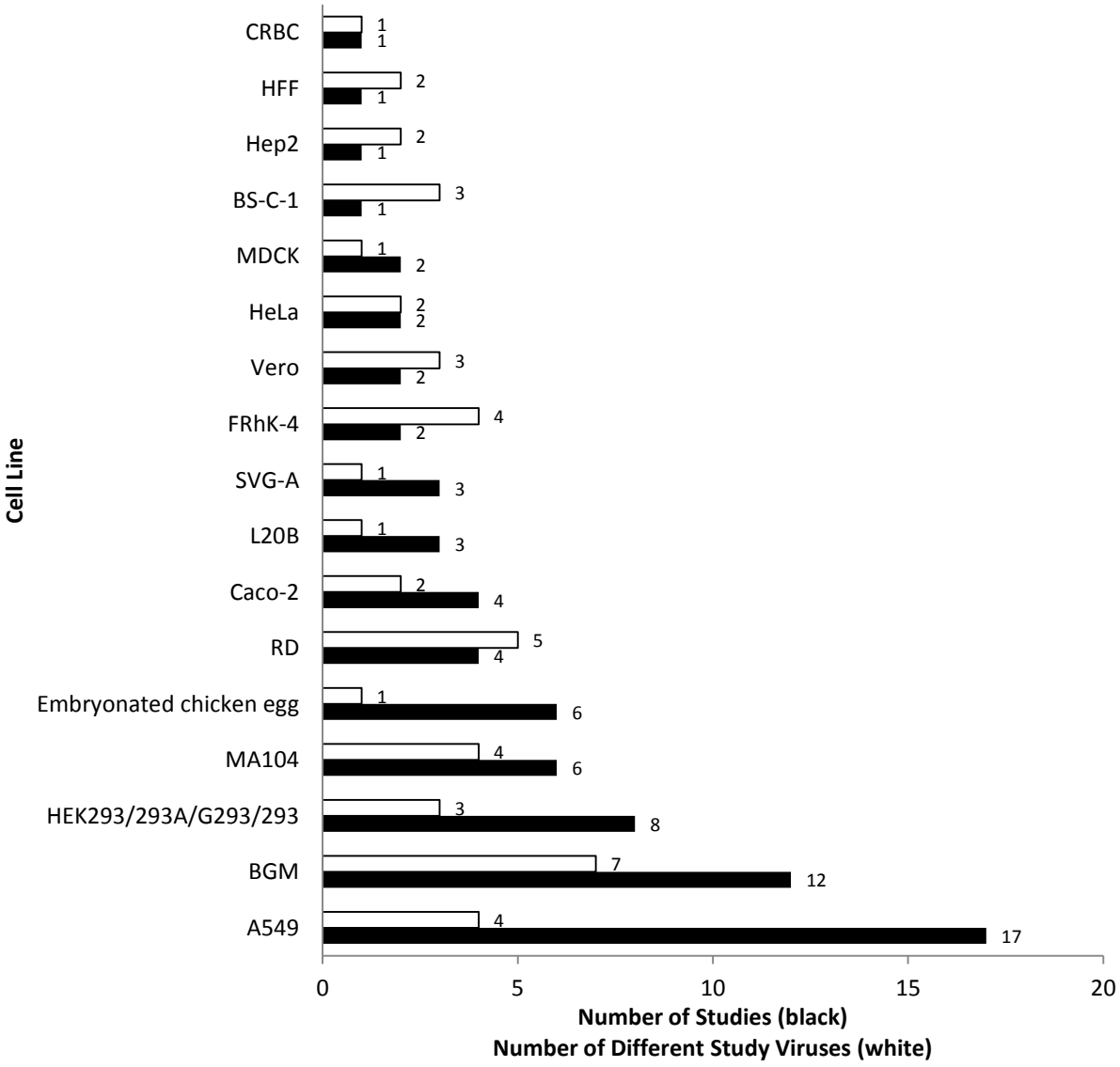

Figure 4. Number of studies that employ each of the different cell lines (black), and the number of different virus cultured on each of the different cell lines (white). Multiple cell lines and viruses per research article were common.

Different viruses and different strains of the same virus are not cultured equally well on the same cell lines; an issue that not only can influence the results of infectivity tests, but also greatly impacts study design, most of which seek to minimise the need for additional cell lines with the associated costs. Moreover, the variable permissiveness (i.e., susceptibility to

infection) of a cell line to different viruses and strains might make cross-study comparisons difficult to interpret, where the same virus is examined using different cell culture lines.

The majority of studies seeking to quantify virus infectivity examine viruses originating from wastewater (n=18), laboratory-derived water, e.g., deionized water (n=17) and surface water, e.g., rivers, springs, lakes/lagoons (n=19; see Figure 5). The large numbers of studies employing waste and river water are indicative of the main research driver—the contamination of bathing waters from sewage effluent. The relatively large numbers of studies using deionized water or other artificial water can be explained by the significant number of method development studies, where the authors seek to minimise some of the confounding environmental factors to better understand method efficacy (i.e., differing water chemistries, pollutants, organic matter).

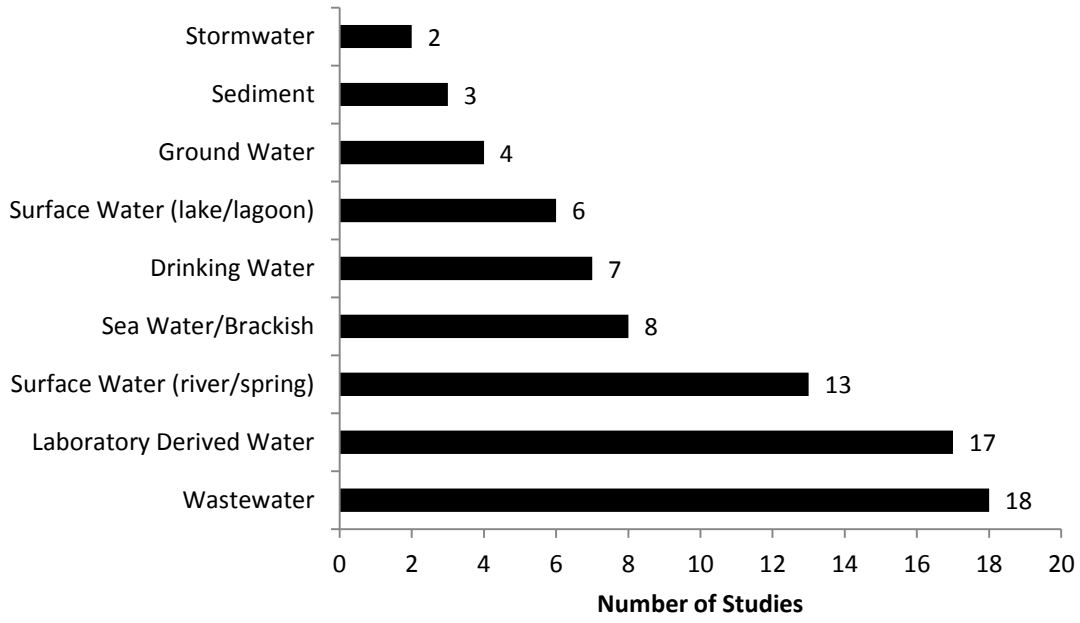

Figure 5. Number of studies that examine virus infectivity recovered from different sources of water. Multiple water sources per research article was common. A full list of media can be found in Appendix A: Table A3.

Conclusions

Key findings

The quantification of infectious viruses in bathing waters has been demonstrated for only a small number of viruses, primarily: human adenovirus, influenza virus, poliovirus and enterovirus. Definitive demonstration of viral infectivity is only possible through cell culture-based methods and the use of human volunteers at this time. Cell culture-based methods are typically undertaken using plaque assays, cell culture followed by observation of cytopathic effects (CPE) or by molecular techniques (PCR), the latter of which is by far the most sensitive. The increased sensitivity provided by PCR facilitates a more rapid determination of infectious virus within a sample, a process that can take weeks using the CPE method to days or even hours using molecular tools. Uniquely, molecular methods applied to infected cell lines can confirm the presence of each of the study viruses by the use of strain-specific probes, while also potentially providing an estimate of their abundance within the sample (e.g., Real-Time Quantitative Reverse Transcription PCR).

There are many logistical constraints confronting cell culture-based methods, the greatest of which might be the need for multiple cell lines to examine multiple viruses. Although some cell lines are routinely used for multiple enteroviruses, i.e., A549 and BGM, there is still uncertainty regarding the efficacy of virus culture across different cell lines, as well as the growth of different strains of the same virus on the same cell line. Moreover, many viruses (e.g., norovirus) have a very limited range of cell lines upon which they can be cultured, thereby necessitating several cell lines for any comprehensive water quality monitoring study. It is unlikely that the relative importance of viruses to human health is currently reflected by the distribution of study viruses, and this is likely a function of the difficulty in culturing many human infectious viruses. A non-trivial biohazard is always present when cell culturing human infectious virus. Hence, there are considerable drivers for the development of alternatives to the cell-culture based approach.

A significant number of papers explore structural integrity-based measures of potentially-infectious virus. These approaches use molecular tools to examine the structural integrity of the viral capsid, antigens and genome. These approaches offer insight into the potential for

isolated viruses to be infectious, but cannot be conclusive, as they are rarely validated against cell culture. Studies only employing structural integrity-based measures of infectivity are still relatively few in number, comprising less than half of the studies in the literature since 2010. Sustained interest in the use of structural integrity-based measures is likely aided by the now widespread use of molecular tools in most laboratories, as well as the significant savings it offers on: cost, labour intensity and equipment required. Additional motivations for using structural integrity-based measures include: safety and the ease at which they can be applied across all human viruses (i.e., no need for virus-specific cell lines). The combination of benefits afforded by the structural integrity-based approach makes it highly attractive to large scale monitoring studies, and given the methodological and financial limitations of implementing cell-culture assays for routine monitoring purposes, the academic and regulatory communities should evaluate the use of structural integrity assays. However, as they remain unproven for predicting virus infectivity, significant additional research and validation against cell culture is required to help justify structural integrity assays as a replacement for cell culture-based assays. Moreover, the statistical uncertainties associated with these structural integrity-based measures need to be quantified before the methodology can be used as the primary method for informing policy.

Implications for policy and practice

Quantification of infectious virus is currently provided using cell culture-based methods. However, these methods are not well suited for large-scale water quality monitoring because of the time, cost and resources required to analyse for several different viruses using a wide range of cell lines over, sometimes, an extended period of time (days to weeks).

Structural integrity-based methods for estimating viral infectivity offer numerous advantages over the cell culture-based methods, as previously described. The structural integrity-based methodology is further strengthened by the use of multiple structural integrity-based assays on a sample, each of which can help to further justify the final estimate of potentially infectious virus within a sample. Results from structural integrity-based methods can be informative, and given the relative ease and cost-effectiveness at which they can be conducted, should not be discarded outright as a tool for informing policy. The combined use of viral coat and genome integrity assays could be used as a tool for high throughput screening of bathing waters for potentially infectious viruses, and, where such estimates

indicate areas of concern, this can be followed up with cell culture-based studies. This scenario could run concurrently with a concerted effort by the research community to validate the structural integrity assays against cell culture. However, the structural integrity-based methods have not been fully validated through inter-laboratory trial against cell culture-based methods, hence, they do not yet offer a demonstrable way forward as a means for quantifying risk to human health in bathing waters.

Typically, the number of human viruses in environmental water samples is very low, necessitating sample concentration prior to analysis. The methods used for concentrating viruses from many litres to several millilitres or microlitres are time consuming and do not lend themselves to routine high intensity sampling of the environment over time and space. All downstream analysis of virus infectivity is constrained by the quality and efficacy of upstream concentration steps—not only in the ability to concentrate samples with minimal or predictable measures of loss, but also in the ability to maintain viral integrity and minimise the influence of contaminants/inhibitors on infectivity assays. The technical and logistical hurdles presented by sample concentration are out of scope for this QSR, but are of the highest importance for the provision of reliable estimates of infective virus.

Implications for research

Innovation in the methods used for virus cell culture is on-going, typically, with an aim to improve on the speed and sensitivity of the assay; in particular:

1) the use of molecular methods for the rapid and highly sensitive detection of a reproducing virus within a cell line (e.g., PCR, qPCR and quantitative-rtPCR);
2) the application of novel reporters and instrumentation for the rapid and highly sensitive detection of virus replication in a cell line (e.g., green fluorescent proteins (GFP), fluorescently-labelled antibodies, cell-sorting); and
3) the identification of novel cell lines upon which to grow infectious virus (e.g., norovirus infection of human B cells).

Continued improvements in speed and sensitivity of cell culture-based methods further strengthen this methodological approach; however, innovations that preclude the need for traditional cell culture will be among the most desired for application to large scale monitoring studies. Molecular tools, innovations in synthetic biology and lab-on-a-chip might

now be able to overcome many of the major bottlenecks, such as: limited host cell susceptibility and real-time reporting of infectious virus—challenges, if overcome, could revolutionise virus monitoring.

In the absence of paradigm-shifting innovation, structural integrity measures can still offer valuable insight. Given that the best available technology for determining virus infectivity (i.e., cell culture) is currently not scalable to large-scale monitoring campaigns, there is a research need to explore and validate a wide range of potentially useful structural integrity methods. Such a research focus would require significant investment, coordination across multiple laboratories and, likely, an international collaboration, given the dispersed expertise of scientists in this discipline.

As previously noted, any progress in the methods used to determine viral infectivity will be constrained by the efficacy and reproducibility of the preceding method of virus concentration. In conclusion, future innovation in methods of virus concentration and viral infectivity would benefit from an increased focus on maximizing the scalability while both minimizing and quantifying the uncertainties.

Competing interests

Financial competing interests – The authors have been commissioned and funded by DEFRA to carry out this research.

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APPENDIX A

Table A1. Viruses that were assayed for infectivity using one of the methods denoted in Table A2. Code for reference numbers can be found on subsequent page.

Human **adenovirus** HAdV 2, 5, 40, 41, C serotype 2, 6, F1	**Norovirus** GI.1, GII.4, WA	**Enterovirus**	**Poliovirus** PV-1 strain LSc-1, Sabin 1, 2ab, 3	**Echovirus 7, 11, 30**	**JC-Polyomavirus** (JCPyV Mad-4)	**Reovirus**	**Aichi virus**	**Avian influenza A H1N1, H5N1, H5N2**	**Hepatitis A (HAV) strain HM175**	**Hepatitis E (HEV)**	**Coxsackie virus A9, B2, B5, 30**	**Rotavirus G1, Wa, SA11 groupA**	**Astrovirus serotype 4**
1, 3, 7, 8, 9, 10, 11, 13, 16, 17, 18, 20, 26, 30, 33, 34, 35, 36, 37, 38, 40, 44, 45, 48, 50	27, 37, 39, 41, 42, 45, 46, 47	1, 3, 5, 6, 7, 14, 20, 32, 45	4, 5, 6, 19, 20, 24, 26, 31, 32, 37, 44	1, 2, 5, 36, 37	8, 9, 10	7	7	12, 15, 21, 22, 23, 25, 43	11, 26, 45	18	5, 26, 36, 37	26, 28, 29, 36, 49	41

Table A2. Methods used to determine virus integrity and infectivity. Code for reference numbers numbers can be found on subsequent page.

Cell Culture-Based Methods					**Viral Integrity-Based Methods**	
Plaque assay	CPE	Human infection	Culture + PCR* (ICC-PCR)	Non-PCR Infection Reporters	Genome integrity	Capsid integrity
8, 11, 16, 17, 19, 24, 25, 28, 29, 37	2, 3, 4, 8, 10, 12, 20, 26, 31, 33, 36, 44, 45	42	1, 4, 5, 6, 7, 8, 9, 11, 13, 15, 16, 20, 21, 28, 29, 32, 35, 38, 40, 48	5, 8, 9, 11, 18, 19, 26, 30, 50	27	10, 11, 12, 14, 16, 18, 21, 22, 23, 24, 27, 34, 37, 39, 41, 42, 43, 46, 47, 49

*Any technique that follows cell culture with the verification and/or quantification of infectious virus by: PCR, qPCR, rt-PCR and/or rt-qPCR

Table A3. Water sources used in virus integrity and infectivity assays. Code for reference numbers can be found on subsequent page.

Laboratory Water (deionized, artificial env.)	Wastewater	Surface water (river, spring)	Surface water (lake/lagoon)	Ground water	Sediment/mud	Stormwater	Sea water/brackish	Drinking water
4, 8, 9, 10, 12, 18, 19, 24, 26, 27, 34, 36, 37, 41, 47, 49, 50	1, 2, 3, 5, 6, 13, 14, 18, 20, 28, 29, 35, 38, 40, 44, 45, 46, 49	3, 7, 8, 13, 15, 16, 17, 29,35, 36, 38, 43, 48	12, 15, 16, 17, 25, 29	7, 17, 33, 42	21, 22, 23	13, 19	10, 11, 13, 15, 32, 43, 44, 48	13, 16, 17, 22, 23, 28, 31

Table A4. Cell lines used to determine virus infectivity and the viruses that were examined for each cell line. Codes for viruses denoted below Table; codes for references from which the data was acquired can be found on subsequent page.

Cell Line	**RD**	**Hep 2**	**A549**	**Caco-2**	**MA104**	**BS-C-1**	**Vero**	**HEK293, 293A, G293 & 293**	**BGM**	**L20B**	**SVG-A**	**FRhK-4**	**MDCK**	**Embryonated chicken egg**	**CRBC**	**HeLa**	**HFF**
Virus	a, b, c, d, e	a, b	a, c, d, e	k, m	a, c, e, l	c, e, l	c, e, l	a, c, e	a, b, c, d, e, h, i	c	f	a, c, g, l	j	j	j	c, e	c, e
Reference	1, 2, 5, 6	1	3, 8, 9, 10, 11, 13, 16, 17, 20, 26, 30, 33, 35, 44, 45, 48, 50	4, 18, 41, 43	4, 26, 28, 29, 36, 49	26	26, 32	9, 20, 26, 30, 34, 35, 38, 40	3, 4, 7, 14, 19, 20, 24, 26, 31, 36, 37, 50,	5,6, 20	8, 9, 10	11, 26	12, 25	12, 21, 22, 23, 25, 43	15	26, 44	26

CODE for viruses in Table A4.

a = Human Adenovirus F, 2, 5, 41

b = Echovirus 2, 7, 30

c = Poliovirus Sabin Type 1, 2ab, 3

d = Enterovirus

e = Coxsackievirus A & B strains

f = JC polyomaviruses Mad-4

g = Hepatitis A virus strain HM175

h = Reovirus

i = Aichi virus

j = Avian influenza H1N1, H5N1, H5N2, H7N1

k = Hepatitis E

l = Human rotavirus G1, Wa, SA11*. Rotavirus SA11 was included for one study as it demonstrated a unique methodology, applicable to other relevant viruses (Yang *et al.*, 2011)

k = Astrovirus serotype 4

Reference numbers used in Tables A1 through A4.

1. (Amdiouni, Faouzi et al. 2012)
2. (Amdiouni, Maunula et al. 2012)
3. (Aslan, Xagoraraki et al. 2011)
4. (Balkin and Margolin 2010)
5. (Battistone, Buttinelli et al. 2014)
6. (Battistone, Buttinelli et al. 2013)
7. (Betancourt, Kitajima et al. 2014)
8. (Calgua, Barardi et al. 2011)
9. (Calgua, Carratala et al. 2014)
10. (Correa, Carratala et al. 2012)
11. (Correa, Souza et al. 2012)
12. (Deboosere, Horm et al. 2011)
13. (Dong, Kim et al. 2010)
14. (Donia, Bonanni et al. 2010)
15. (Dovas, Papanastassopoulou et al. 2010)
16. (Fongaro, Nascimento et al. 2013)
17. (Garcia, Viancelli et al. 2012)
18. (Girones, Carratala et al. 2014)
19. (Cantera, Chen et al. 2010)
20. (Hewitt, Leonard et al. 2011)
21. (Horm, Deboosere et al. 2011)
22. (Horm, Gutierrez et al. 2012)
23. (Horm, Gutierrez et al. 2012)
24. (Kim, Katayama et al. 2011)
25. (Lebarbenchon, Yang et al. 2011)
26. (Lee, Lee et al. 2013)
27. (Li, De Keuckelaere et al. 2014)
28. (Li, Gu et al. 2010)
29. (Li, Gu et al. 2011)
30. (Li, He et al. 2010)
31. (Mahalanabis, Reynolds et al. 2010)
32. (Ming, Zhu et al. 2011)
33. (Ogorzaly, Bertrand et al. 2010)
34. (Ogorzaly, Bonot et al. 2013)
35. (Ogorzaly, Cauchie et al. 2013)
36. (Pang, Lee et al. 2012)
37. (Parshionikar, Laseke et al. 2010)
38. (Polston, Rodriguez et al. 2014)
39. (Richards, Watson et al. 2012)
40. (Rodriguez, Polston et al. 2013)
41. (Sano, Pinto et al. 2010)
42. (Seitz, Leon et al. 2011)
43. (Shoham, Jahangir et al. 2012)
44. (Silverman, Peterson et al. 2013)
45. (Simmons and Xagoraraki 2011)
46. (Tian, Yang et al. 2012)
47. (Wang and Tian 2014)
48. (Wyn-Jones, Carducci et al. 2011)
49. (Yang, Gu et al. 2011)
50. (Dunams, Sarkar et al. 2012)

APPENDIX B

1. European Patents Office search

The European Patent office database was used for this search (this contains information on patents registered worldwide) http://worldwide.espacenet.com/advancedSearch?locale=en_EP, which is suggested at https://www.gov.uk/search-for-patent.

The following methodology was applied:

1. A search of the **titles and abstract** was first carried out. The search terms combinations applied were based on the search terms used in the main literature review:
   (detection or detect or monitor or monitoring or extract or extraction) AND (infectious or infective or infectivity or viable or inactiv or intact or integrity) AND (virus or viral or phage) AND water*
   As the patents database did not allow more than 10 search terms at a time, we undertook three separate searches using the following terms here (number of results provided in brackets hereafter):

- *detect* and infect* or viable or inactiv* or intact or integrity and virus or viral or phage and water (108)*
- *monitor* and infect* or viable or inactiv* or intact or integrity and virus or viral or phage and water (12)*
- *extract* and infect* or viable or inactiv* or intact or integrity and virus or viral or phage and water (298)*

2. The above three lists were collated and duplicates were removed (resulting in 395 patents).
3. All entries with a publication date of 2000 or before were removed (resulting in 277 patents). The reasoning behind this was that any successful/relevant methods with an application made before 2001 would have most likely appeared by now in the scientific literature. A similar approach has been followed in the literature review.
4. The patents list was further refined by excluding all titles which included one or more of the following terms - these terms (after a first review of the list) were deemed to indicate the patent was not relevant to this study (this process resulted in a list of 100):
   antibod*, antiviral, bovine, canine, chicken, cucumber, cure, diagnose, disinfectant, drug, duck, HIV, influenza, inhibit*, medicine, pig, porcine, poultry, prawn, prevention, swine, tomato, treatment, tumour, vaccin*, vegetable.

5. Some simpler searches for keywords in the **title only** were then undertaken to ensure relevant patents had not been overlooked. The search term combinations used were as follows:

   detect* and virus and water in the title (7)
   monitor* and virus and water in the title (0)
   extract* and virus and water in the title (2)
   detect* and viral and water in the title (1)
   monitor* and viral and water in the title (0)
   extract* and viral and water in the title (1)
   These results were checked for duplicates (none found) and removed earlier than patents 2001 (4).

   The list from steps 4 and step 5 (107 in total, no duplicates) were finally collated, making an assessment on its relevance to this review. 15 patents were found to be **potentially relevant** to this review, and their abstracts were reviewed in more detail. The selected patents are listed in Table B1 below.

6. Closer review of the abstracts indicated that all patents selected were either not relevant to this study or the methods they described were already covered in the literature (see Table B1 for further details on this review for each selected patent).

Table B1. Key information on summary of patents search results

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	Application number	Country of application	Organisation (if applicable)	Key points	Target virus	Target organism/sample type	Is the patent of relevance to this study?	link	Further review if found to be relevant/potentially relevant
DETECTION METHOD OF VIRUS CONTAMINATION FROM ENVIRONMENT WATER	KR20000034564	South Korea	not stated	reverse transcription multiplex nested PCR	Adeno virus and enteric viruses, but states that can be applied to other 'generalised' viruses	unclear - patent refers to contamination from 'environment water'. A further search found a publication by the same first author, and this suggests the method can be applied to environmental samples (see http://www.nrcresearchpress.com/doi/abs/10.1139/w00-014#.VNT6KaBF3cs)	Yes	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20020105&CC=KR&NR=20020000280A&KC=A	Unlikely to be relevant
PROBE FOR DETECTION OF ENTERIC VIRUS DETECTION KIT AND METHOD FOR ENTERIC VIRUS WITH THE SAME	WO2002KR01530	South Korea	BIOMEDLAB CORP	oligonucleotide probe	enteric virus	Patent description refers to clinical samples, or to monitoring water contaminated with water-borne enteric viruses - although it is unclear if it has already been applied to such as samples	Potentially	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20030220&CC=WO&NR=03014397A1&KC=A1	Not relevant as does not address infectivity
METHOD FOR DETECTION OF HEPATITIS A VIRUS RNA IN WATER AND SALIVA BY RT-PCR METHOD	RU20050135515	Russia	FEDERAL NOE G UCHREZHDENIE NAU	method to abate inhibition of RT-PCR when detecting HAV RNA - hence increasing effectiveness, sensitivity and reliability of detection methods	Hepatitis A	concentrates of river water and saliva	Yes	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=1&ND=3&adjacent=true&locale=en_EP&FT=D&date=20070927&CC=RU&NR=2307168C2&KC=C2	Not relevant as does not address infectivity
METHOD FOR DETECTION OF COAGULANT'S ANTI-VIRUS ACTIVITY DURING WATER CONDITIONING	UA20060013605U	Ukraine	MARZEIEV INSTITUTE OF HYGIENE AND MEDICAL ECOLOGYOF ACADEMY OF MEDICAL SCIENCES	detection of coagulants antivirus activity in eluent - based on decrease in bacteriophages number	intestinal bacteriophages	eluent	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20070425&CC=UA&NR=22767U&KC=U	NA

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	Application number	Country of application	Organisation (if applicable)	Key points	Target virus	Target organism/sample type	Is the patent of relevance to this study?	link	Further review if found to be relevant/potentially relevant
METHOD AND APPARATUS FOR RECOVERING MICROORGANISM	JP20080049496	Japan	METAWATER CO LTD	Apparatus for recovering microorganisms (including viruses) - using antibody (bonded to stirring bar) which bonds with the detection target, then filtration.	Patent refers to protozoa, bacterium, virus, etc.	unclear - patents refers to 'water treating process'	Potentially		Conceptually already covered in literature - unlikely to be relevant
METHOD FOR DETECTION OF MINIMUM AMOUNTS OF HEPATITIS A VIRUS IN WATER OBJECTS	RU20080125383	Russia	not stated	RT-PCR/dilution	Hepatitis A	water	Probably not - unclear about method details	http://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20091210&DB=EPODOC&locale=en_EP&CC=RU&NR=2375455C1&KC=C1&ND=4	Not relevant as does not address infectivity
Quick test paper for detecting enterovirus and method for preparing same	CN20081147353	China	not stated		enterovirus EV71 and Coxsackievirus A16 viruses	unclear	Possibly - but unclear if applicable to environmental samples	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20100217&CC=CN&NR=101650366A&KC=A	Unlikely to be relevant
METHOD FOR DESALINATION OF SEAWATER	US20100851387	USA	GLOBE PROTECT, INC	method and an apparatus for the modification of seawater, brine, or alike, to render it suitable for irrigation/industrial use, including deactivation of viruses	not stated	not stated	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20101202&CC=US&NR=2010300973A1&KC=A1	NA

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	Application number	Country of application	Organisation (if applicable)	Key points	Target virus	Target organism/sample type	Is the patent of relevance to this study?	link	Further review if found to be relevant/potentially relevant
METHOD AND APPARATUS FOR DESALINATION OF SEAWATER	WO2010US44910	USA	GLOBE PROTECT, INC	Method and an apparatus for the modification of seawater, brine, or alike, to render it suitable for irrigation, industrial use, or for further treatment in a drinking water purification plant - inactivated viruses by means of high-intensity macrosound.	not stated	not stated	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20120216&CC=WO&NR=2012021122A1&KC=A1	NA
PCR detection kit and detection method of epizootic haematopoietic necrosis virus (EHNV)	CN201015114 94	China	THIRD INST OCEANOGRAPHY SOA	PCR detection kit and a detection method of epizootic haematopoietic necrosis virus	epizootic haematopoietic necrosis virus	not stated, but this virus affects salmon and trout	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20110119&CC=CN&NR=101948937A&KC=A	NA
RT-PCR (reverse transcription-polymerase chain reaction) detection method of aquatic bicistronic virus	CN201113157 27	China	ZHEJIANG INSTITUTE OF FRESH WATER FISHERIES)	RT-PCR (reverse transcription-polymerase chain reaction) detection method of aquatic bicistronic virus	aquatic bicistronic virus genome	not stated	Possibly - but unclear if applicable to environmental samples	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20120125&CC=CN&NR=102329896A&KC=A	Not relevant as does not address infectivity and does not refer to human viruses
RT-LAMP (reverse transcription loop-mediated isothermal amplification) detection kit and RT-LAMP detection method for SVCV (spring viremia of carp virus)	CN201214030 53	China	YANGTZE RIVER FISHERIES RESEARCH INSTITUTE, CHINESE ACADEMY OF FISHERY SCIENCES	RT-LAMP (reverse transcription loop-mediated isothermal amplification) detection kit and a RT-LAMP detection method for SVCV (spring viremia of carp virus).	spring viremia of carp virus	fish	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20130116&CC=CN&NR=102876811A&KC=A	NA

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	Application number	Country of application	Organisation (if applicable)	Key points	Target virus	Target organism/sample type	Is the patent of relevance to this study?	link	Further review if found to be relevant/potentially relevant
Real-time fluorescent quantitative PCR (polymerase chain reaction) detection kit for shrimp white spot syndrome virus	CN201214945 25	China	(TIANJIN AQUATIC ANIMAL DISEASE CONTROL AND PREVENTION CONTROL CENTER	real-time fluorescent quantitative PCR (polymerase chain reaction) detection kit for shrimp white spot syndrome virus	white spot syndrome virus	shrimp	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20130306&CC=CN&NR=102952902A&KC=A	NA
VIRAL TRAPPERS	WO2013IN00165	USA and India	KATRAGADDA BHASKARA RAO	Extract of tephrosia Purpurea arial parts supplemented with immuno boosters such as chromium and zinc. Viral trappers is a very effective tool to fight intractable HIV/AIDS.	HIV, cold, cold sores, hepatitis B and other opportunistic infections	humans	No	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20140123&CC=WO&NR=2014013503A1&KC=A1	NA
Device for separating and extracting virus in water and method for extracting sapovirus in water	CN201412341 28	China	INSTITUTE OF MICROBIOLOGY, HEILONGJIANG ACADEMY OF SCIENCES	Method for extracting the sapovirus in the water comprises the following steps: filtering a water sample, eluting with a beef extract solution, treating by adopting an isoelectric point sedimentation method and then centrifuging, remelting and carrying out secondary concentration, and then extracting nucleic acid, so that extraction of the sapovirus is completed.	sapovirus	water	Potentially	http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20140813&CC=CN&NR=103981084A&KC=A	Not relevant as only refers to concentration of samples

2. Engineering and Physical Sciences Research Council (EPSRC) grants database search

The EPSRC Grants on the Web database was used for this search: http://gow.epsrc.ac.uk/NGBODefault.aspx

The database notes that it 'provides information about research and training grants supported by EPSRC' and allows to 'search the entire Grants on the Web database'. The website also highlights that 'this search now includes the Key Findings and Impact Summaries of funded projects, as well as suggested potential use(s) and relevant sectors.'

The search term used in the literature review did not yield any results, so a simpler (wider) expression was used, derived from the yellow-highlighted part of the literature search expression below.

(detection or detect or monitor or monitoring or extract or extraction) AND (infectious or infective or infectivity or viable or inactiv or intact or integrity) AND (virus or viral or phage) AND water*

Therefore, the search terms used for the GoW data base were (number of results in brackets; see Table B2 for further details):

- Virus and water (14)
- Viral and water (6)
- Phage and water (0)

As the search expression was very wide, it was decided to undertake a first filtering based on the title and, if required, a review of the abstract. Based on this filtering, all grants identified were deemed not relevant to this study. The reason for excluding each grant was recorded in a brief note on the attached excel sheet (see Table B2) – e.g., 'Dendrons for Targeted Gene Therapy', excluded because it relates to the medical field. Note that the search also yielded various 'lists', such the list of 'EPSRC Support by Research Area in Chemical Structure' - only results relating to grant proposals were considered and included in the count above.

Table B2 Key information on EPSRC grant proposal search results

Title	Relevant to this study? "first filter" (based on title/abstract)	Excluded from further review as it refers to:
Search terms: " virus and water"		
Poly-y-glutamic acid as a template for functionalised water-soluble biomaterials	*No*	Biology research proposal
Strategies for Quantifying and Controlling Free Living Nematode Populations and Consequent Damage by Tobacco Rattle Virus to Improve Potato Yield and	*No*	Virus on potatoes
Large Scale Lattice Boltzmann for Biocolloidal Systems	*No*	Biology research proposal
New Strategies for Sampling, Analysing and Understanding Aerosols	*No*	Aerosols
Is a NOESY-like 2 dimensional experiment possible in mass spectrometry?	*No*	Mass spec method development
Development of a self-disinfecting dental alginate impression material	*No*	Dental field
Centre for Secure Information Technologies - Tranche 2 Proposal	*No*	IT viruses
Chemical Vapour Deposition for the Generation of Visible Light Activated Antimicrobial Coatings	*No*	Refers to antimicrobial coating
Dendrons for Targeted Gene Therapy	*No*	Medical
Infrastructure and the 21st century infectious diseases	*No*	Engineering/infrastructure field
Supramolecularly assembled functional nanocages	*No*	Field of synthetic chemistry
Development of new injectable, PolyGeneCaP composites for gene therapy	*No*	Medical
Elucidating the photochemistry of inorganic nanostructures	*No*	Materials physics
Detecting infectious organisms: A concerted approach using genomics, molecular engineering and nano-enabled bio-MEMS technologies (AptaMEMS-ID)	*No*	Medical
Search terms: "viral and water"		
Development of a self-disinfecting dental alginate impression material	*No*	Dental field
Dendrons for Targeted Gene Therapy	*No*	Medical
Centre for Secure Information Technologies - Tranche 2 Proposal	*No*	IT virus
Thermodynamic and Structural Characterisation of Membrane Peptides and Proteins	*No*	Biological chemistry - method development on lipids/protein interaction

Title	Relevant to this study? "first filter" (based on title/abstract)	Excluded from further review as it refers to:
Structure-based rational design of oligonucleotide-mediated chemical ribonucleases	*No*	Medical
Development of new injectable, PolyGeneCaP composites for gene therapy	*No*	Medical

3. Natural Environment Research Council (NERC) grants database search

The NERC Grants on the Web database was used for this search:
http://gotw.nerc.ac.uk/freetext.asp

The database notes that it 'holds information about the current and past research projects, and masters and doctoral training grants which are funded by the Natural Environment Research Council through various awards". For consistency with the EPSRC grants search (see above), the following search expressions were used (number of results in brackets; see Table B3 for further details):

- Virus and water (5)
- Viral and water (13)
- Phage and water (3)

As for the EPSRC search, as the search expression was very wide, it was decided to undertake a first filtering based on the title and, if required, a review of the abstract. If past the first filter, the grant information was further assessed for relevance to the study.
This review did not provide any results of relevance to this study or any results which were not already covered in the literature reviewed. The exception to this is a large study, with multiple partners, titled "New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum"; the project will commence in April 2015 (running until 2018). After contacting the investigators of this project, it was made clear that there was to be no measure of infective virus in this study.

Table B3 Key information on NERC grant proposal search results

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	Relevant to this study? First 'filter' (based on title/abstract)	Further information if past 'first filter'			Further assessment of relevance if past 'first filter'
		Key details	Organisation	Award period	
Search terms: Virus and water					
A UK Network for Environmental and Food Virology	Potentially relevant	Study aimed to develop integrated approaches to tackling existing issues regarding food and environmental transmission of viral pathogens, and integrated responses to resolve any new problems or study emerging diseases.	Food & Environment Research Agency -FERA, Plant Pest & Disease	1 Apr 2007 - 31 Mar 2008	Potentially relevant but as study completed 6 years ago, anticipated that any significant outputs regarding methodology will be available in the peer-review literature.
Effects of host population size and isolation on parasite dynamics in a naturally fragmented host metapopulation	No - refers to rodents and their parasites.				
Lambda integrase gene family: markers for phage diversity, environmental regulation, and gene transfer in freshwater bacteria.	No - project refers to phages in freshwater bacteria, and considered questions such as what is the relationship between such phages and other genetic elements?				
Manufactured Nanoparticle Migration in Groundwaters	No - project dealt with project is with determining the mobility of metal oxide nanoparticles in groundwater.				
The ecology of protist associated human pathogens.	Potentially relevant	Study aimed to elucidate the abundance and diversity of a range of amoeba associated pathogens in different polluted and unpolluted environments.	University of Warwick, Biological Sciences	11 Jun 2011 - 10 Sep 2012	

Title (green=relevant/potentially relevant [required further	Relevant to this study? First 'filter' (based on title/abstract)	Further information if past 'first filter'			Further assessment of relevance if past 'first filter'
Search terms: Viral and water					
A UK Network for Environmental and Food Virology	Potentially relevant	Aimed to develop integrated approaches to tackling existing issues regarding food and environmental transmission of viral pathogens, and integrated responses to resolve any new problems or study emerging diseases.	Food & Environment Research Agency -FERA, Plant Pest & Disease	1 Apr 2007 - 31 Mar 2008	Potentially relevant but as study completed 6 years ago, anticipated that any significant outputs regarding methodology will be available in the peer-review literature.
Biogeochemical cycling of N-osmolytes in the surface ocean	No -the study investigated nitrogen-containing compounds, including glycine betaine (GBT), choline and trimethylamine N-oxide (TMAO), in marine organisms. In the marine environments, these compounds are frequently released into the sea water due to the change of environmental conditions, such as viral attack or grazing.				
Biogeochemical cycling of N-osmolytes in the surface ocean	same as above				
Biogeochemical cycling of N-osmolytes in the surface ocean	same as above				
Cyanophage as engines of gene transfer within populations of cyanobacteria.	No - study dealt with a variety of molecular methodologies to assess the role of viral-mediated gene transduction in determining the genetic structure of Baltic Sea Nodularia populations.				
Lambda integrase gene family: markers for phage diversity,	No - project refers to phages in freshwater bacteria, and considered				

Title (green=relevant/potentially relevant [required further	Relevant to this study? First 'filter' (based on title/abstract)	Further information if past 'first filter'			Further assessment of relevance if past 'first filter'
environmental regulation, and gene transfer in freshwater bacteria.	questions such as what is the relationship between such phages and other genetic elements?				
New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum	Yes	The project will design and test experimental and modelling tools to permit the robust recovery and quantification of viral populations from contrasting matrices (e.g. seawater, freshwater, sediments, effluent, shellfish).	University of Cambridge, Pathology	1 Apr 2016 - 31 Mar 2018	Relevant but after contacting the investigators of this project, it was made clear that there was to be no measure of infective virus in this study. Study involves several partners and will start on the 1st April 2015.
New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum	same as above		Centre for Env Fisheries Aqua Sci CEFAS, CEFAS Weymouth Laboratory	1 Apr 2015 - 31 Mar 2018	
New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum	same as above		NERC Centre for Ecology and Hydrology, Emmett	1 Apr 2015 - 31 Mar 2018	
New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum	same as above		Bangor University, Sch of Environment and Natural Resources	1 Apr 2015 - 31 Mar 2018	
New approaches for the quantitative detection of human pathogenic viruses within the freshwater-marine continuum	same as above		University of Liverpool, Institute of Integrative Biology	1 Oct 2015 - 30 Sep 2017	

Title (green=relevant/potentially relevant [required further	Relevant to this study? First 'filter' (based on title/abstract)	Further information if past 'first filter'			Further assessment of relevance if past 'first filter'
The ecology of protist associated human pathogens	Potentially relevant	The study aimed to elucidate the abundance and diversity of a range of amoeba associated pathogens in different polluted and unpolluted environments.	University of Warwick, Biological Sciences	11 Jun 2011 - 10 Sep 2012	See above
Management and Control of Taste and Odour Problems associated with Geosmin and 2-MIB in Drinking Water Supplies	No - deals understanding the drivers of elevated VOC production (Geosmin and 2-MIB) in drinking water reservoirs within the Wessex Water catchment.the work includes identifying conditions that lead to the exudation of VOCs with a specific focus on the role(s) of viral lysis.				
Search terms: Phage and water					
Cyanophage as engines of gene transfer within populations of cyanobacteria.	No - study dealt with a variety of molecular methodologies to assess the role of viral-mediated gene transduction in determining the genetic structure of Baltic Sea Nodularia populations.				
Diversity of indigenous bacteriophage from Antartic and Southern Ocean waters.	No -study investigated transposable elements in bacteria from Antarctic water to provide a 'snap shot' of the phage in Antarctic and Southern Ocean water (plaque assays and electron microscopy were used to count numbers; study also included examination of morphology, host range and transduction potential).				

Title (green=relevant/potentially relevant [required further	Relevant to this study? First 'filter' (based on title/abstract)	Further information if past 'first filter'			Further assessment of relevance if past 'first filter'
Lambda integrase gene family: markers for phage diversity, environmental regulation, and gene transfer in freshwater bacteria.	No - project refers to phages in freshwater bacteria, and considered questions such as what is the relationship between such phages and other genetic elements?				

4. International Organisation for Standardisation (ISO) review

The ISO standards catalogue relating to the microbiology of waters was reviewed; this can be found at:
http://www.iso.org/iso/iso_catalogue/catalogue_ics/catalogue_ics_browse.htm?ICS1=07&ICS2=100&ICS3=20
The list contains 27 ISO standards. A first filtering of the list was undertaken based on the ISO standard title – for example many standards referred to bacterial cultures not relevant to the study and were therefore excluded from further review. Based on this first review, 20 standards were found to be not relevant to this study. The remaining 7 were deemed 'potentially relevant' and reviewed further. This entailed examining the ISO description, where this was available, as well as relevant literature referring to the ISO standard where applicable. This further review indicated that none of the ISO standards were directly relevant to this study. Key information relating to the ISO review is provided in Table B4.

Table B4 Key information on ISO search results

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	**"First filter"**	**Further details if past "first filter"**	**Further assessment of relevance if past "first filter"**
Water quality -- Enumeration of culturable micro-organisms -- Colony count by inoculation in a nutrient agar culture medium	Not relevant to this study		
Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 1: Method by enrichment in a liquid medium	Not relevant to this study		
Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 2: Method by membrane filtration	Not relevant to this study		
Water quality -- Evaluation of membrane filters used for microbiological analyses	Not relevant to this study		
Water quality -- Detection and enumeration of intestinal enterococci -- Part 1: Miniaturized method (Most Probable Number) for surface and waste water	Not relevant to this study		

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	**"First filter"**	**Further details if past "first filter"**	**Further assessment of relevance if past "first filter"**
Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method	Not relevant to this study		
Water quality -- General guidance on the enumeration of micro-organisms by culture	Not relevant to this study		
Water quality -- General guidance on the enumeration of micro-organisms by culture	Not relevant to this study		
Water quality -- Enumeration of Escherichia coli and coliform bacteria -- Part 1: Membrane filtration method for waters with low bacterial background flora	Not relevant to this study		
Water quality -- Enumeration of Escherichia coli and coliform bacteria -- Part 2: Most probable number method	Not relevant to this study		
Water quality -- Detection and enumeration of Escherichia coli and coliform bacteria -- Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of E. coli in surface and waste water	Not relevant to this study		
Water quality -- Practices for evaluating and controlling microbiological colony count media used in water quality tests	Not relevant to this study		
Water quality -- Detection and enumeration of bacteriophages -- Part 1: Enumeration of F-specific RNA bacteriophages	Potentially relevant	Specifies a method for the detection and enumeration of F-specific ribonucleic acid bacteriophages. The sample is incubated with an appropriate host strain. Applicable to all kinds of water, sediments and sludges, even for shellfish extracts. The presence of F-specific RNA bacteriophages in a water sample indicates pollution by wastewater contaminated by human or animal faeces.	Not directly relevant to the study as refers to bacteriophages.

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	**"First filter"**	**Further details if past "first filter"**	**Further assessment of relevance if past "first filter"**
Water quality -- Detection and enumeration of bacteriophages -- Part 2: Enumeration of somatic coliphages	Potentially relevant	No further details available on this page	Not directly relevant to the study as refers to bacteriophages.
Water quality -- Detection and enumeration of bacteriophages -- Part 3: Validation of methods for concentration of bacteriophages from water	Potentially relevant	ISO 10705-3:2003 specifies the general principles for assessing the performance of methods for the concentration of bacteriophages from water. Concentration is recommended for those water samples expected to contain < 3 pfp (plaque-forming particles) per millilitre. Concentration methods can be applied to all kinds of water provided that the amount and nature of suspended solids and/or dissolved matter do not interfere with the concentration procedure. ISO 10705-3:2003 does not give specific details of concentration methods, but outlines the fundamental principles for evaluating the suitability of a particular method for a given type and volume of water. Annex A gives examples of methods that have been found satisfactory and their fields of application.	Not directly relevant to the study as refers to bacteriophages.
Water quality -- Detection and enumeration of bacteriophages -- Part 4: Enumeration of bacteriophages infecting Bacteroides fragilis	Potentially relevant	No further details available on this page	Not directly relevant to the study as refers to bacteriophages.
Water quality -- Detection and enumeration of Legionella	Not relevant to this study		
Water quality -- Enumeration of Legionella	Not relevant to this study		
Water quality -- Detection and enumeration of Legionella -- Part 2: Direct membrane filtration method for waters with low bacterial counts	Not relevant to this study		

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	**"First filter"**	**Further details if past "first filter"**	**Further assessment of relevance if past "first filter"**
Water quality -- Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR)	Not relevant to this study		
Water quality -- Guidance on validation of microbiological methods	Potentially relevant	No further details available on this page	Not directly relevant to the study as refers to method validation/performance testing.
Water quality -- Requirements for establishing performance characteristics of quantitative microbiological methods	Potentially relevant	No further details available on this page	Not directly relevant to the study as refers to method validation/performance testing.
Water quality -- Enumeration of Clostridium perfringens -- Method using membrane filtration	Not relevant to this study		
Water quality -- Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water	Not relevant to this study		
Water quality -- Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods	Potentially relevant	ISO 17994:2014 specifies an evaluation procedure for comparing two methods with established performance characteristics according to ISO/TR 13843 and intended for the quantification of the same target group or species of microorganisms. It provides the mathematical basis for the evaluation of the average relative performance of two quantitative methods against chosen criteria for the comparison. It does not provide data for assessment of the precision of the methods being compared. It is appropriate that the precision of methods is assessed as part of their performance characterization. ISO 17994:2014 does not provide methods for the verification of method performance characterization in a single laboratory.	Not directly relevant to the study as refers to method validation/performance testing.

Title (green=relevant/potentially relevant [required further review]; orange=not relevant)	**"First filter"**	**Further details if past "first filter"**	**Further assessment of relevance if past "first filter"**
Water quality -- Detection and enumeration of thermotolerant Campylobacter species	Not relevant to this study		
Water quality -- Detection of Salmonella spp.	Not relevant to this study		